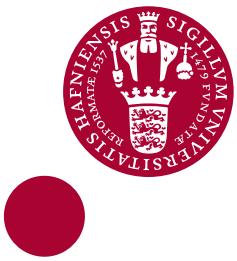

Protecting the Aging Genome

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Published in:
Trends in Cell Biology

DOI:
10.1016/j.tcb.2019.12.001

Publication date:
2020

Document version
Publisher's PDF, also known as Version of record

CellPress
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Review

Protecting the Aging Genome

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Mounting evidence suggests that DNA damage plays a central role in aging. Multiple tiers of defense have evolved to reduce the accumulation of DNA damage, including reducing damaging molecules, repairing DNA damage, and inducing senescence or apoptosis in response to persistent DNA damage. Mutations in or failure of these pathways can lead to accelerated or premature aging and age-related decline in vital organs, supporting the hypothesis that maintaining a pristine genome is paramount for human health. Understanding how we cope with DNA damage could inform on the aging process and further on how deficient DNA maintenance manifests in age-related phenotypes. This knowledge may lead to the development of novel interventions promoting healthspan.

Highlights

DNA damage accumulates with aging.

Defects in DNA repair lead to premature aging.

Emerging drugs that target DNA repair may alleviate age-associated phenotypes.

From Increased DNA Damage to Aging

Multiple endogenous and exogenous molecules can chemically modify our DNA. To deal with these stressors, cells have developed ways to reduce the production of, or eliminate, endogenous damaging molecules before damage occurs (Box 1), to repair damage once it occurs, or to eliminate cells that have accumulated too much damage (Figure 1). These three tiers of defense are the focus of this review. The most well-described mechanism to reduce toxic molecules is antioxidant removal of reactive oxygen species (ROS) before they can react with other molecules such as DNA, proteins, or lipids. In addition, oxidized lipids and proteins can react and form toxic adducts with DNA [1,2]. Nonenzymatic antioxidants such as glutathione and vitamin C and E as well as antioxidant enzymes such as superoxide dismutase, catalase, and peroxidases attempt to counter these reactive molecules and could protect the genome. If this first tier of defense fails, repair enzymes coordinate the processes that attempt to reverse the damage and return the DNA to its undamaged (functional) state. These highly conserved repair mechanisms can be classified into the following pathways: direct reversal, base excision repair, nucleotide excision repair, double-strand break repair, and interstrand crosslink repair (Box 2).

The association between DNA damage and aging is well established with extensive data from humans and animal models showing increased markers of genome instability with age [3,4]. One possible reason for an age-associated increase in DNA damage is that DNA repair capacity may decrease with age [5–8]. Markers of DNA damage have been observed in age-associated diseases such as dementias, cardiovascular disease, and cancer, suggesting that genome instability could be a causal factor in these pathologies [9–11]. A compelling piece of evidence for a causal role of DNA damage is the observation that some patients with inherited defects in DNA repair proteins show features of premature or accelerated aging (Figure 2) [12,13]. Importantly, defects in different pathways lead to aging features in different tissues. For example, individuals with Cockayne syndrome and ataxia-telangiectasia display features of premature neurological aging [14,15], while Werner syndrome and Hutchinson–Gilford progeria patients display features of cardiovascular aging [16,17]. Due to the significant clinical heterogeneity between the diseases, the impact of some genes on processes outside DNA repair and the observation that none of the diseases perfectly phenocopies human aging, the diseases are often called segmental progerias. More than 50 DNA repair disorders have been described with various degrees of overlapping phenotypes with aging, such as neurodegeneration, cancer, and cardiovascular diseases (Table 1, Key Table) [12]. This could suggest that different types of DNA damage contribute to different pathologies in aging (Figure 3).

DNA Damage and Age-Related Disease

One of the most visible consequences of DNA damage with age is seen in sun-exposed skin. Here, UV light induces two main mutagenic lesions in the DNA – cyclobutane pyrimidine dimers and 6–4 pyrimidine pyrimidone – that contribute to an age-associated increased risk of cancer development [5].

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Box 1. Endogenous Sources of DNA Damage

Oxidative stress is not the only metabolic byproduct that can damage DNA. Complex lesions can occur by a multitude of other processes. For example, acetaldehyde, formed as a byproduct of acetyl metabolism or after alcohol consumption, readily reacts with DNA forming a variety of single-base adducts that can further react to form highly toxic interstrand DNA crosslinks [165]. An important way to deal with this stress is through enzymatic removal of acetaldehyde by the enzyme acetaldehyde dehydrogenase that converts this molecule into acetate. Accordingly, point mutations in the ALDH2 gene that encodes the acetaldehyde dehydrogenase lead to increased susceptibility to alcohol-induced cancers [166]. Interestingly, the dehydrogenation of alcohol to acetaldehyde is reversible with the equilibrium pointing heavily towards alcohol, and acetaldehyde levels, even in the case of intoxication, hover in the micromolar range while ethanol concentrations remain 100-fold higher. Conversely, acetaldehyde dehydrogenation to acetate is essentially irreversible and acetate levels reach millimolar levels during intoxication. Thus, cells may have developed biochemical processes that attempt to minimize the amount of acetaldehyde present in cells perhaps to limit the genotoxic effect of these metabolites.

Another source of endogenous DNA damage is single-base methylation facilitated by *S*-adenosylmethionine (SAM). SAM is an important molecule that acts as a physiological methylation donor in various enzymatic reactions such as CpG island methylation in our genome thereby regulating gene expression. However, SAM can also nonenzymatically react with DNA and thereby induce mutagenic DNA methyl adducts [167,168] that need to be repaired through the direct reversal pathway as indicated in Box 2. Interestingly, SAM is synthesized from methionine and adenosine and dietary methionine restriction has been shown to reduce SAM levels [169] as well as extend the lifespan of multiple organisms [170]. One speculative hypothesis is thus that methionine restriction could reduce spontaneous mutagenesis in our genome by lowering SAM levels, a phenomenon that has been observed in bacteria [167,168]. Accordingly, decreasing SAM levels increases *Drosophila* and *Caenorhabditis elegans* lifespans [171,172].

In sum, processes removing genotoxic molecules have evolved and their absence or dysfunction can lead to pathologies associated with aging.

These types of DNA damage are normally repaired by nucleotide excision DNA repair and patients with defects in this pathway are sensitive to sunlight, often developing accelerated skin aging as exemplified by xeroderma pigmentosum, where cancer risk is 10 000-fold increased (Figure 3A) [18].

UV exposure also leads to the formation of oxidative stress that can additionally be generated from endogenous sources such as the mitochondria [19]. The most common oxidative DNA lesion is the mutagenic 8-oxoguanine, a type of DNA damage that has been shown to accumulate in several tissues with age. 8-Oxoguanine is repaired through base excision repair, which removes the damaged base and, in the process, induces a break in the DNA strand containing the damage base. Patients with defects in base excision repair, particularly in the steps involving DNA breaks, often develop neurodegeneration [20]. Notably, single- and double-strand DNA breaks activate the enzyme poly-ADP-ribose (PAR) polymerase 1 (PARP1), and the activity of this enzyme increases with age suggesting that strand breaks accumulate in the elderly [21]. Excessive activation of PARP1 can lead to depletion of the substrate NAD^+ resulting in altered intracellular changes in redox homeostasis, increased lactate production, and a reduction in the molecule acetyl-CoA. This results in wide-ranging changes in cellular metabolism, with, for example, alterations in neurotransmitters and myelin synthesis [22] (Figure 3B). PARP1 activation is seen in several age-associated neurodegenerative diseases [23] where markers of double-strand DNA breaks (53BP1 and gammaH2AX) are also observed. These two markers are among the most consistently increased with age across multiple tissues as well as in senescent cells, suggesting that double-strand DNA breaks may accumulate with age [4,24].

Oxidative DNA base damage may also contribute to an age-associated increase in point mutations, which is observed in multiple tissues including nonreplicating tissues such as neurons in the brain [25]. Nevertheless, patients with inherited defects in the mismatch DNA repair pathway that specifically fixes mutagenic misincorporated bases are highly cancer prone, yet do not develop neurodegeneration [26] (Figure 3C). It is therefore still unclear what point mutations might contribute to aging.

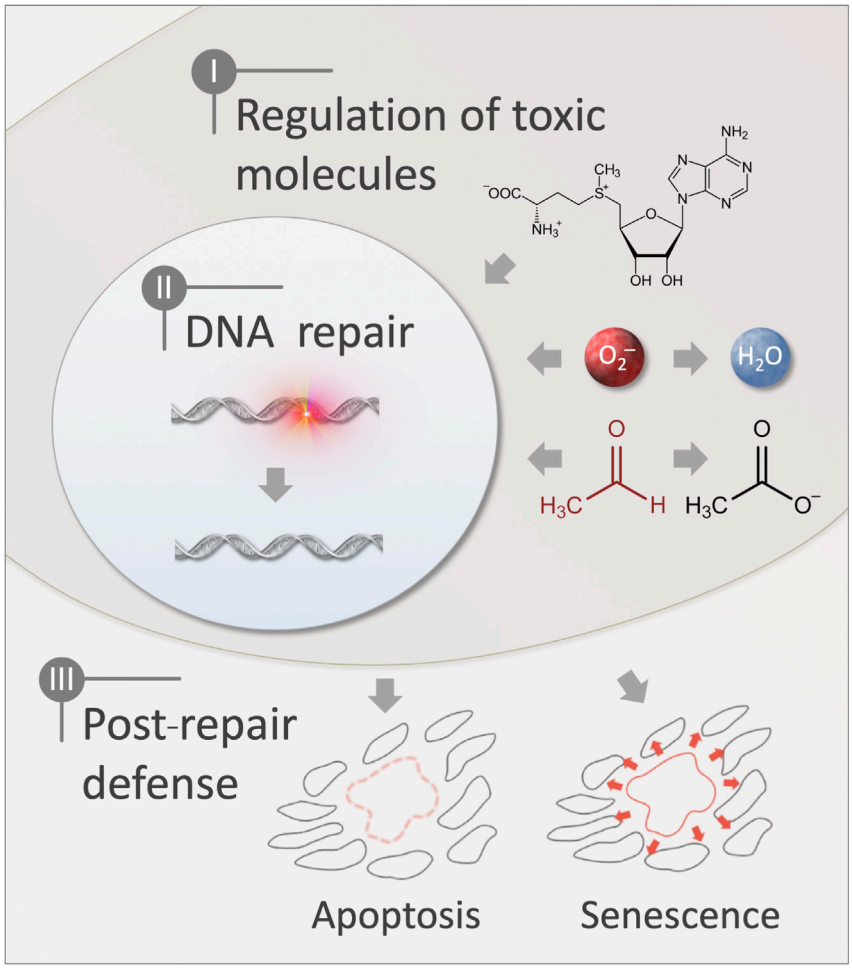

Figure 1. Three Tiers of Defense against DNA Damage.
(I) Regulation of toxic molecules such as free radicals, reactive acyls, and *S*-adenosyl methionine limit the amount of damage that occurs in DNA. (II) DNA repair attempts to correct damage that may occur as a result of either endogenous or exogenous DNA damage, but also as a result of normal cellular DNA metabolic processes such as DNA replication. (III) If repair fails and damage accumulates, the cell may activate programs leading to permanent cell cycle arrest, termed senescence, or the induction of programmed cell death through apoptosis.

In addition to the direct influence of mutagenesis on replicating tissues is the potential for DNA damage to lead to replication stalling and induction of cell death. This has been suggested to be an underlying pathogenic outcome in certain DNA repair diseases; for example, Fanconi anemia, Bloom syndrome, and Werner syndrome, where hypogonadism, anemia, and hair loss are prevalent [16,27,28]. These features are also seen in normal aging [29–31] and it is tempting to speculate that these features could be caused by similar issues with replication, particularly in stem cells (Figure 3D).

Consequences of Unrepaired Genetic Damage

If repair fails and damage accumulates, three outcomes can occur: cells can transform and become cancerous, cells can enter a nonproliferating state termed senescence, or cells can die through, for example, apoptosis. Notably, all of these outcomes are altered with age [32–34].

Cell Death

A form of programmed cell death, apoptosis is necessary for normal cell turnover and is essential to a plethora of other biological processes. Apoptosis can be executed via Bcl-2 activation of caspases, via signals from the death receptor on the plasma membrane, or via induction by granzyme B secreted from cytotoxic T cells (Tc cells) [35]. Endonucleases and proteases are activated by active caspases, eventually leading to the death of the cell. With age, however, apoptotic activity changes. In heart [36], kidney [37], skeletal muscle [38], and Tc cells [39], increased apoptosis has been reported, perhaps contributing to loss of cellularity in these tissues. This escalation across various tissues may be attributed to the increased production of free radicals [40] and furthermore exacerbated by the accumulation of DNA damage in the aged cells [41]. As the risk increases for cells to turn cancerous and dysfunctional with advancing age, increased apoptosis in aged cells is argued to be a defense strategy. In other tissues, such as the colon, apoptosis appears to decrease with age perhaps contributing to the accumulation of senescent cells and age-associated carcinogenesis [42].

Parthanatos, another form of cell death, is particularly interesting because DNA damage is central to its initiation. Here, activation of the DNA damage responder PARP1 is the initiating event that leads to the formation PAR polymers, activation of apoptosis-inducing factor (AIF), and caspase-independent cell death [43]. Parthanatos has been implicated in age-associated neurodegeneration, particularly Parkinson's disease, a disorder where DNA damage has been shown to occur [23]. Accordingly,

Box 2. Mammalian DNA Repair Pathways

DNA repair in general is a three-step process: damage detection, damage removal, and resynthesis of new DNA (Figure I). Direct reversal repair deals with the removal of simple base modifications without altering the base or backbone of the DNA and is primarily used in repairing damage from DNA-alkylating agents. This process occurs with two major types of proteins: *O*6-methylguanine-DNA methyltransferases (MGMTs) using a single repair reaction where the methyl group is transferred to the MGMT protein thereby inactivating it; and repair via AlkB dioxygenases using an iron-catalyzed multistep repair reaction. Mutations in these enzymes have been associated with increased brain, lung, and bladder cancer risk [173,174] perhaps due to the mutagenic nature of the *O*6-methylguanine lesion.

Base excision repair deals with single-base modifications where the damaged base is recognized and removed and one, in short-patch repair, or several, in long-patch repair, new undamaged bases are added instead. Defects in this process have most commonly been linked with neurodegeneration and cancer [175], two central pathologies in aging.

Mismatch DNA repair deals with misincorporated bases during replication or after post-replicative DNA synthesis as part of other DNA repair pathways. The classic genetic mismatch repair disease is Lynch syndrome where DNA mutations accumulate in the rapidly proliferating cells in the gastrointestinal tract resulting in a high risk of colon cancer development [26].

Nucleotide excision repair corrects bulkier and/or helix-distorting lesions, often caused by UV irradiation, that require the removal of a piece of single-stranded DNA, an oligonucleotide, containing the DNA damage. Accordingly, patients with inherited defects in nucleotide excision repair suffer from sun sensitivity and increased risk of skin cancer development [111,176]. In addition, neurodegeneration and short stature are common features, although the pathogenesis for these particular traits is still debated [177].

Homologous recombination is one of two pathways that attempt to repair double-strand DNA breaks. The process relies on homologous chromosomes that occur during S or G2/M phase in the cell cycle. The enzymatic steps involve detection of the break, resection of the 5′ end of the DNA, and invasion of the single-stranded DNA in the sister chromatid after which elongation of the invaded DNA can occur to allow bridging of the area of DNA that was broken. Inherited deficiency in homologous recombination can lead to a long list of phenotypes including neurodegeneration, microcephaly, ionizing radiation sensitivity, short stature, cancer, anemia, immune deficiency, skeletal defects, pigmentation changes, and hypogonadism [125,178].

A second major pathway that attempts to correct double-strand DNA breaks is nonhomologous end joining. This process entails no, or very minor, resection of the 5′ DNA end followed by simple ligation of the DNA ends. Inherited deficiencies in nonhomologous end joining most prominently lead to immunodeficiency due to the role that nonhomologous end joining has in DNA recombination at antibody loci. In addition, patients can display microcephaly, short stature, anemia, ionizing radiation sensitivity, cardiovascular disease, skeletal defects, and immune deficiency [119].

One of the most complex lesions to occur in our cells is the interstrand crosslink, where the two complementary DNA strands are covalently connected. Here, the lesion is detected and a strand is incised on each side of the crosslink allowing the crosslinked base to be flipped out of the helix in what is called an unhooking step. Resynthesis by a translesion polymerase allows bridging of the gap. Subsequent removal of the unhooked crosslinked base is done by nucleotide excision repair. Inherited deficiency in the repair of interstrand crosslinks typically results in Fanconi anemia, which primarily affects rapidly proliferating cells in the bone marrow eventually leading to bone marrow failure and pancytopenia [27]. In addition, patients with Fanconi anemia can suffer from microcephaly, short stature, neurodegeneration, cancer, skeletal defects, and skin pigmentation changes [143,179].

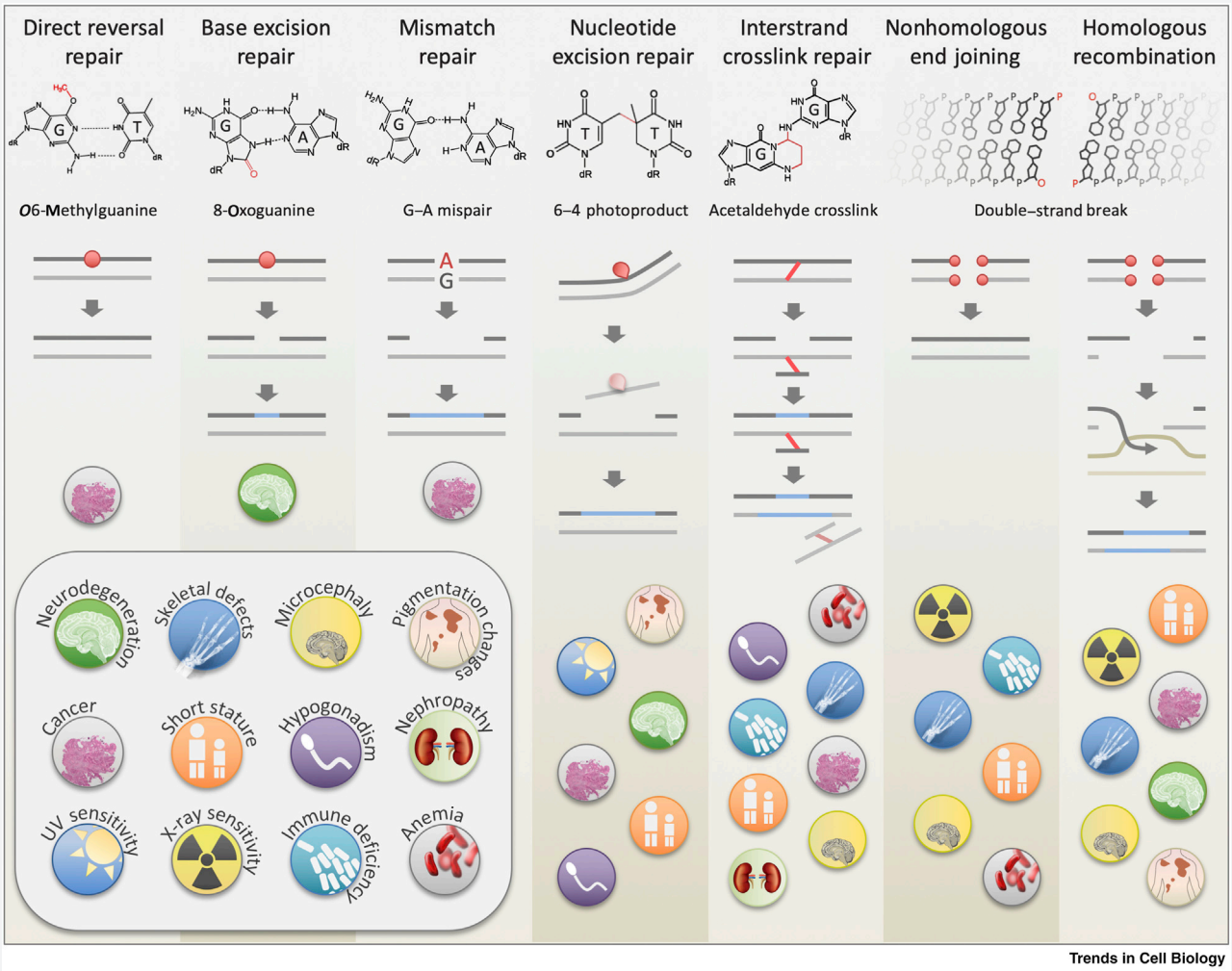

Figure I. Different Pathways Deal with Different Lesions.

Direct reversal repair attempts to correct single-base methylation events. Base excision repair corrects single-base lesions such as guanine oxidation. Nucleotide excision repair deals with bulky or helix-distorting lesions such as 6–4 photoproducts or cyclopyrimidine dimers. Interstrand crosslink repair is required to correct covalently linked DNA strands while double-strand break repair deals with breaks to both strands. A plethora of clinical phenotypes are associated with defects in the different pathways.

markers of double-strand DNA breaks have been found to accumulate in the neurons of Alzheimer's and Parkinson's patients [9,44], suggesting that age-associated DNA breaks could lead to neurodegeneration through parthanatos. Parthanatos has also been implicated in chronic heart failure [45] and diabetes [46].

Cell Senescence

If a cell evades death on DNA damage, induction of senescence can occur instead, allowing a cell to linger in an unhealthy and proinflammatory state [47]. A cell can enter senescence from various stimuli, such as replicative senescence in somatic cells, oncogene-induced senescence, and excessive DNA damage. At younger ages, senescence contributes to tumor suppression, wound healing, and tissue development; however, with age these cells accumulate and are likely to contribute to

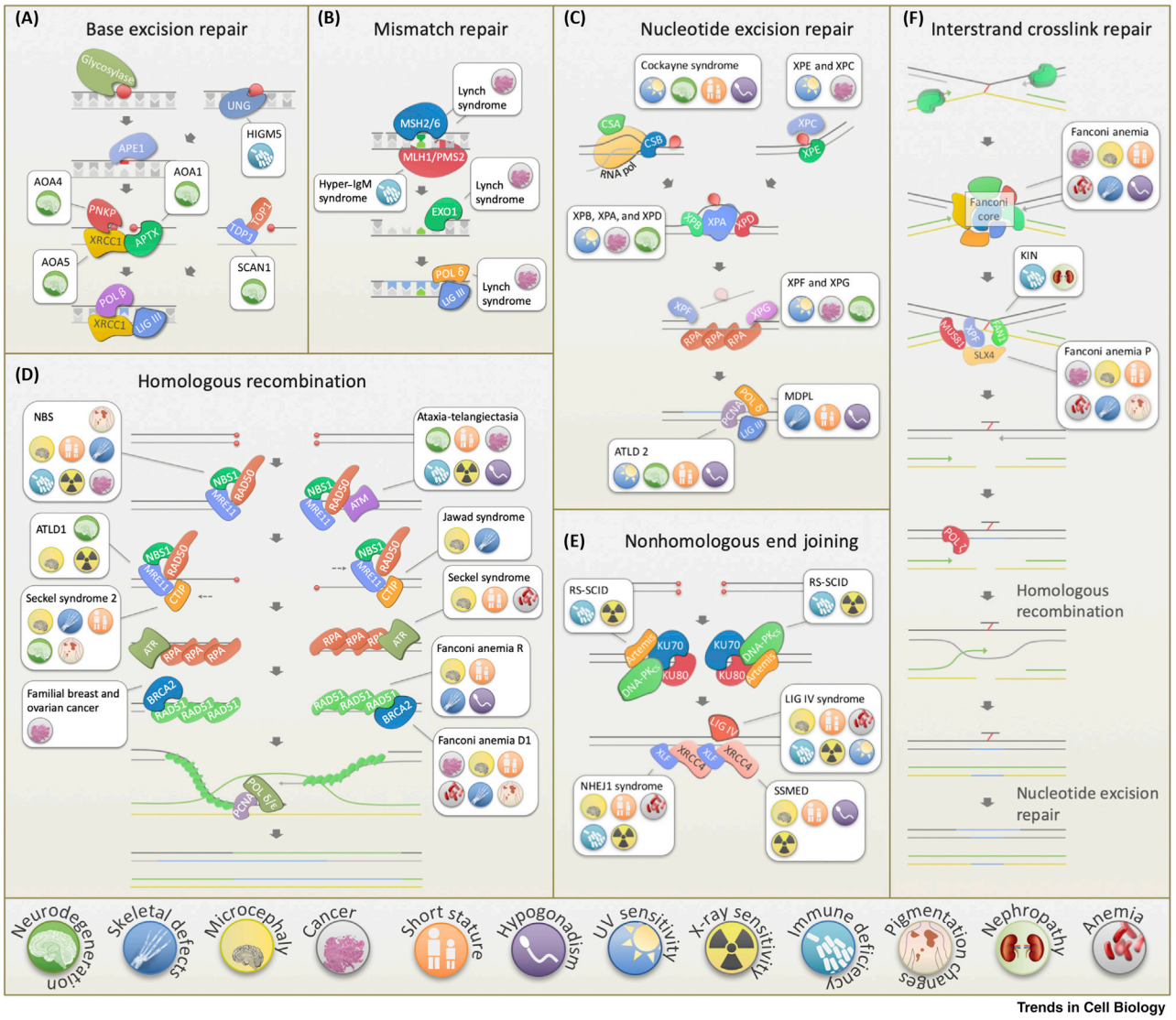

Figure 2. An Overview of Clinical Features Associated with Mutations in Specific Proteins.

Abbreviations: AOA, ataxia oculomotor apraxia; ATLD, ataxia telangiectasia-like disorder; HIGM, immunodeficiency with hyper-IgM; KIN, karyomegalic interstitial nephritis; LIG IV, Ligase IV; MDPL, mandibular hypoplasia, deafness, progeroid features, and lipodystrophy syndrome; NBS, Nijmegen breakage syndrome; NHEJ1, nonhomologous end joining factor 1; RS-SCID, radiation-sensitive severe combined immunodeficiency; SCAN1, spinocerebellar ataxia with axonal neuropathy-1; SSMED, short stature, microcephaly, and endocrine dysfunction.

many pathologies [48]. Senescent cells adopt a proinflammatory secretome termed the senescence-associated secretory phenotype [47], increasing the secretion of inflammatory cytokines and contributing to a proinflammatory microenvironment. Overall, p53, p21, and p16 are involved in inducing senescence in response to excess DNA damage [49]. Signaling from the DNA damage site, and not the damage itself, appears to be involved in the entry into senescence after reaching a threshold level of genotoxic stress [24]. Accordingly, point mutations as seen in mismatch repair deficiencies that do not activate the canonical DNA damage response are not associated with senescence. Conversely, DNA breaks or helix-distorting lesions that activate the DNA damage response induce senescence [24,50]. These observations suggest that the DNA damage response may drive degenerative processes, such as neurodegeneration and sarcopenia, while point mutations may go

Key Table

Table 1. DNA repair Disorders and Their Associated Phenotypes

Pathway	Disease	Gene[a]	Phenotype
Base excision repair	Ataxia-oculomotor apraxia 1 [92]	APTX (AR)	
	Ataxia-oculomotor apraxia 4 [93]	PNKP (AR)	
	Ataxia-oculomotor apraxia 5 [20]	XRCC1 (AR)	
	Familial adenomatous polyposis 3 [94]	NTHL1 (AR)	
	Immunodeficiency with hyper IgM 5 [95]	UNG (AR)	
	Machado–Joseph disease [96]	ATXN3 (AD)	
	Microcephaly, seizures, and developmental delay [97]	PNKP (AR)	
	Spinocerebellar ataxia with axonal neuropathy 1[98]	TDP1 (AR)	
Mismatch repair	Immunodeficiency with hyper IgM [99,100]	PMS2, MLH1 (AR)	
	Lynch syndrome [101]	MSH2, MLH1 (AD)	
Nucleotide excision repair	Cerebro-oculofacioskeletal syndrome [102]	CSB, ERCC1, XPD, XPG (AR)	
	Cockayne syndrome [14,103]	CSA, CSB (AR)	
	Trichothiodystrophy [104]	XPD, GTF2H5 (AR)	
	UV-sensitive syndrome [105]	UVSSA (AR)	
	Xeroderma pigmentosum, group A, B, D, F, G [106–110]	XPA, XPB, XPD, XPF, XPG (AR)	
	Xeroderma pigmentosum, group C, E and V [111–114]	XPC, XPE, POLH (AR)	
	Xeroderma pigmentosum–Cockayne syndrome complex [115]	XPB, XPD, XPF, XPG (AR)	
Nonhomologous end joining	Early infantile epileptic encephalopathy 28 [116]	WWOX (AR)	
	Childhood-onset epileptic encephalopathy [117]	CDH2 (AD)	
	Ligase IV syndrome [118]	LIG IV (AR)	
	Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation [119]	XLF (AR)	
	Omenn syndrome [120]	RAG1 (AR)	
	Radiosensitive severe combined immunodeficiency [121]	Artemis, DNA-PKcs (AR)	
	Schimke immuno-osseous dysplasia [122]	SMARCAL1 (AR)	
	Short stature, microcephaly, and endocrine dysfunction [123]	XRCC4 (AR)	
Homologous recombination	Ataxia-oculomotor apraxia 2 [124]	SETX (AR)	
	Ataxia-telangiectasia [125–127]	ATM (AR)	

(Continued on next page)

Key Table. ***Continued***

Pathway	Disease	Gene[a]	Phenotype
	Ataxia-telangiectasia-like disorder 1 [128]	MRE11 (AR)	
	Bloom syndrome [28,129,130]	BLM (AR)	
	Fanconi anemia R [131]	RAD51 (AD)	
	Hoyeraal–Hreidarsson syndrome [132]	RTEL1 (XLR)	
	Jawad syndrome [133]	CTIP (AR)	
	Lung disease, immunodeficiency, and chromosome breakage [134]	NSMCE3 (AR)	
	Natural killer cell and glucocorticoid deficiency [135]	MCM4 (AR)	
	Nijmegen breakage syndrome [136–138]	NBS1 (AR)	
	Progressive external ophthalmoplegia 2B [139]	RNASEH1 (AR)	
	Seckel syndrome [140,141]	ATR, ATRIP (AR)	
	Seckel syndrome 2 [142]	CTIP (AR)	
Interstrand crosslink repair	Fanconi anemia [27,143,144]	FANCA-FANCS (AR)	
	Karyomegalic interstitial nephritis [145]	FAN1 (AR)	
	Warsaw breakage syndrome [146,147]	DDX11 (AR)	
Additional or multipathway disorder	Aicardi–Goutières syndrome [148,149]	RNASEH2A, TREX1 (AR/AD)	
	Ataxia-telangiectasia 2 [150]	PCNA (AR)	
	Baller–Gerold syndrome [151]	RECQL4 (AR)	
	Dilated cardiomyopathy 1A [152]	LMNA (AD)	
	Dyskeratosis congenita [153]	DKC1 (XLR)	
	Emery–Dreifuss muscular dystrophy 2 [154]	LMNA (AD)	
	Hutchinson–Gilford progeria syndrome [17]	LMNA (AD/AR)	
	Li–Fraumeni syndrome [155]	TP53 (AD)	
	Mandibular hypoplasia, deafness, progeroid features, and lipodystrophy [156]	POLD1 (AD)	
	Meier–Gorlin syndrome [157,158]	ORC1, ORC4, ORC6, CDT1 (AR)	
	Premature ovarian failure 8 [159]	STAG3 (AR)	
	Rapadilino syndrome [160]	RECQL4 (AR)	
	Rothmund–Thomson syndrome [151,161]	RECQL4 (AR)	
	Ruijs–Aalfs syndrome [162]	SPRTN (AR)	
	Werner syndrome [163,164]	WRN (AR)	

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Key Table. *Continued*

Pathway	Disease	Gene[a]	Phenotype
Key			
Neurodegeneration	Microcephaly	UV sensitivity	Nephropathy
Cancer	Skeletal abnormalities	Hypogonadism	Immunopathy
Short stature	Lung disease	Pigmentation changes	Anemia
X-ray sensitivity	Myopathy	Cardiovasculopathy	

[a]Abbreviations: AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.

undetected without impacting cell growth. Hyperactivation of the DNA damage response is associated with neurodegeneration [9] while hypermutability, such as seen in the mismatch DNA repair-defective disease Lynch syndrome, is associated with cancers [26]. Further supporting this hypothesis is the observation that mutations in p53 or p16 that facilitate senescence signaling are among the most commonly mutated genes in cancers [51,52].

Interestingly, when senescent cells are abolished either through genetic manipulation or via senolytic drugs, biological aging is significantly halted in mice [53,54]. Therefore, trials are now under way to test the ability of senolytics to postpone age-associated pathologies in humans [55]. Notably, multiple drugs are being pursued that either directly or indirectly impact DNA repair or the consequence of DNA damage.

Future Prospects: Developing Interventions through DNA Repair

Given the possible role of DNA damage in multiple age-associated diseases, interventions targeting DNA repair is a major emerging focus for the field. The largest efforts have been on developing drugs that inhibit DNA repair as a means to potentiate chemotherapeutics in oncology. Nevertheless, some pharmaceuticals have been developed that either directly or indirectly augment DNA damage (Figure 4). Only a few molecules have been suggested to directly stimulate DNA repair: RAD51-stimulatory compound 1 (RS-1) increases double-strand DNA repair pathway homologous recombination [56], nicorandil stimulates base excision repair through the APE1 enzyme [57], and aspirin is suggested to stimulate nucleotide excision repair [58]. To our knowledge the only compound to have been tested in lifespan interventions is aspirin, which increases the lifespan of mice, although it is speculative whether this effect is due to stimulation of DNA repair [59].

Other modulators of the DNA damage response appear to impact aging. For example, inhibition of PARP1 leads to lifespan extension in certain model organisms [21]. Concomitant with the age-associated activation of PARP1 is the observation that persistent DNA damage foci containing the

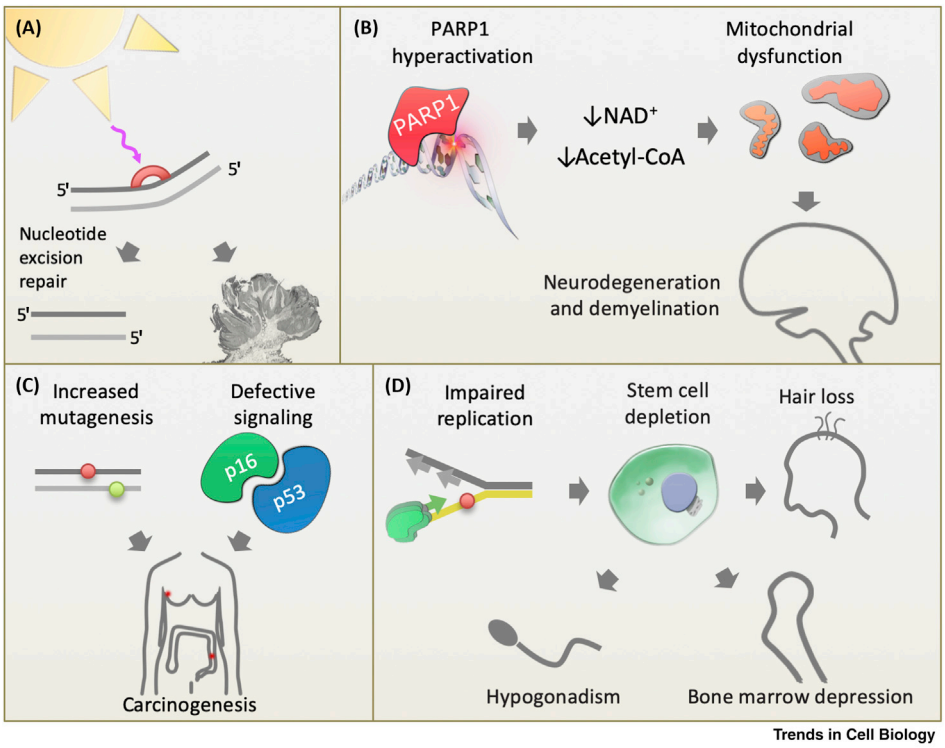

Figure 3. Pathology of DNA Damage.
(A) UV light from sunlight causes mutagenic DNA damage that is normally repaired by nucleotide excision repair but may lead to carcinogenesis with aging. (B) DNA damage, such as strand breaks, leads to poly-ADP-ribose (PAR) polymerase 1 (PARP1) activation and loss of NAD^+ and acetyl-CoA resulting in mitochondrial dysfunction and neurodegeneration. (C) Point mutations, such as seen if mismatch DNA repair is dysfunctional, and loss of tumor suppressors may contribute to age-associated carcinogenesis. (D) Defects in replication or damage-induced replication stalling may contribute to loss of stem cells and dysfunction of replicating tissues such as gonad, hair, and bone marrow.

proteins 53BP1, γH2AX, and FOXO4 accumulate in aging cells [4,60]. Notably, signaling from these foci may contribute to the senescence-associated secretory phenotype [47]. Another approach to tackle this signaling cascade is therefore to break up these foci. Treatment with a FOXO4-mimicking peptide leads to the removal of p53- and FOXO4-containing foci, thus facilitating apoptosis of senescent cells, regrowth of lost hair, and lifespan extension in models of severe premature aging [60].

Weight loss and metabolic changes are common age-associated features and are seen in multiple DNA repair disorders as well as in mouse models of these disorders [14,61,62]. One possible explanation for these phenomena is the observation that persistent DNA damage through PARP1 leads to loss of NAD^+ and consequently changes in the NAD^+:NADH ratio, a master regulator of the intermediary metabolism. Alternatively, increasing levels of the NAD^+-metabolizing enzyme CD38 has been proposed to be involved in the age-associated loss of NAD^+ [63]. Increasing NAD^+ levels could therefore alleviate aspects of aging potentially as a result of persistent PARP1 and/or CD38 activation. A number of studies have recently validated nicotinamide riboside, an NAD^+ precursor, as a potentially effective therapy for premature aging diseases and normal aging through stimulation of DNA repair pathways [21,64–66]. Further, inhibition of CD38 appears to reinstate NAD^+ levels leading to healthier aging in mice [67]. Along these lines, the molecule P7C3 activates NAMPT, the rate-limiting enzyme that converts nicotinamide to nicotinamide riboside, and this activation can be neuroprotective [68].

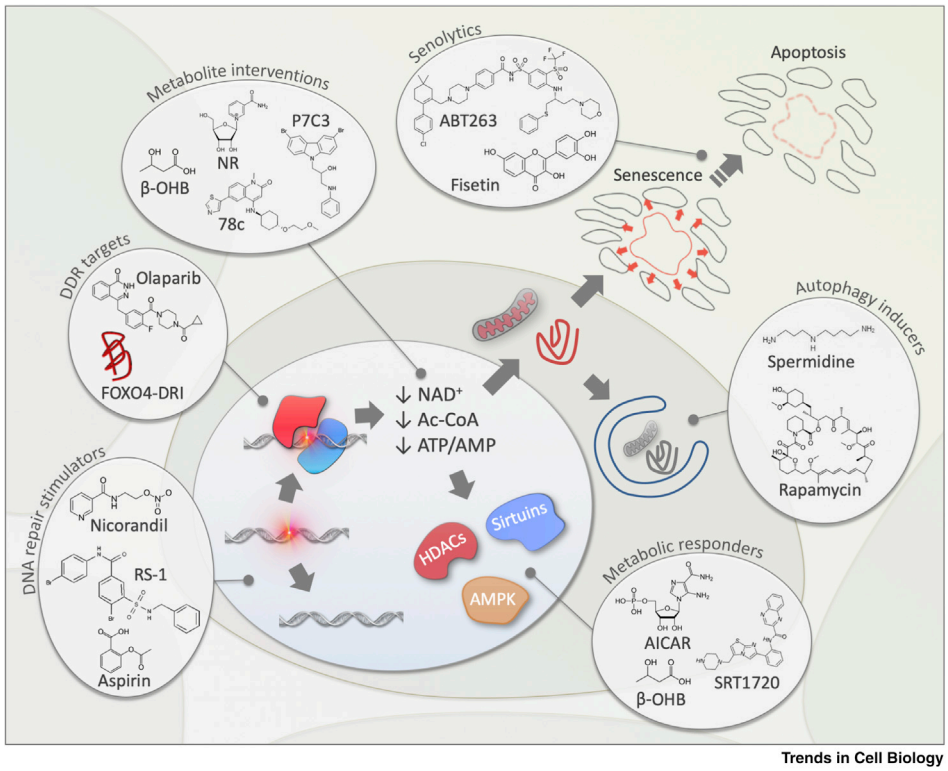

Figure 4. Interventions in the DNA Damage Cascade.
An overview of where interventions can be attempted to ameliorate the consequences of DNA damage, including some examples of compounds. Abbreviations: Ac-CoA; acetyl CoA; AMPK, AMP-activated protein kinase; DDR, DNA damage response; HDAC, histone deacetylase.

Conversely, loss of NAD^+ leads to attenuation of NAD-dependent enzymes. Here, the sirtuin family of protein deacylases may be particularly important. Multiple sirtuins appear to be central in regulating the rate of aging in a variety of species and most mammalian sirtuins stimulate DNA repair. For example, SIRT1 activates base excision repair through XRCC1 [69], nucleotide excision repair through XPA [70], and double-strand break repair through Ku70 and the WRN helicase [71,72]. SIRT2 activates ATRIP and stimulates double-strand break repair [73]. SIRT3 stimulates the base excision repair enzyme OGG1 in mitochondria [74]. SIRT6 stimulates base excision repair and double-strand DNA repair for example through activation of PARP1 [75]. SIRT7 acts on histone 3, lysine 18 acetylation to stimulate nonhomologous end joining [76]. The effect of NAD^+ on genome stability could, therefore, be responsible for the life- and healthspan effects mediated by sirtuins.

Alterations of the NAD^+:NADH ratio lead to shunting of pyruvate to lactate and loss of the small metabolite acetyl-CoA [77]. Ketones generated through a ketogenic diet can act as acetyl-CoA donors and could be efficacious as a premature aging therapy and attenuate the consequences of normal brain aging [64,78]. In addition to the role of β-hydroxy-butyrate as a fuel source, this metabolite can also alter the epigenetic landscape through inhibition of histone deacetylases and attenuates features of age-associated neurodegeneration [78,79].

Another point of intervention is through the enzymatic cascade that responds to metabolic changes. The energetic deficiency that occurs with DNA damage leads to compensatory activation of the AMP-activated protein kinase (AMPK) energy sensor [80,81]. Interestingly, decreasing caloric intake activates AMPK and extends the lifespan from yeast to primates [82–84]. Pharmacological activation of

AMPK by the compound AICAR ameliorates symptoms in models of age-associated neurodegenerative and cardiovascular diseases, conditions where DNA damage is known to accumulate [85,86].

DNA damage also leads to altered mitochondrial function with increased mitochondrial volume and membrane potential likely as a compensatory response to increased energy expenditure [62,80]. The increased membrane potential inhibits mitochondrial degradation via mitophagy and leads to accumulation of damaged mitochondria [62,65]. Accordingly, stimulation of autophagy via mTOR inhibition reduces mitochondrial membrane potential and attenuates mitochondrial dysfunction in premature aging [62,87]. Additionally, loss of proteostasis is seen on DNA damage [88,89] and we speculate that autophagic stimulation, via for example the mTOR inhibitor rapamycin, may be efficacious in ameliorating this as well.

If homeostasis of the cells cannot be remediated post-DNA damage, ultimately cells can enter senescence. Here, senescent cells can be specifically targeted and driven to apoptosis via senolytic compounds without killing nonsenescent cells. Recently recognized senolytics are, for example, ABT263 [90], fisetin [91], and the peptide analog FOXO4-DRI [60]. These drugs appear to attenuate multiple features of aging. In the case of ABT263, rejuvenation of hematopoietic stem cells (HSCs) and senescent muscle stem cells (MuSCs) occurred in both total-body-irradiated and aged mice via depletion of senescent cells. Fisetin extended the health- and lifespan of normally aged mice by reducing senescence in a cell-type-specific manner. Last, FOXO4-DRI dramatically recovered hair loss, fitness, and kidney function in an accelerated aging mouse model ($Xpd^{TTD/TTD}$).

Concluding Remarks

Genome instability plays a significant role in the progression of aging and protecting our aging genomes is therefore of fundamental importance for healthy aging. A major issue for the development of interventions targeting aging is the long trial time and difficulty in determining positive outcomes (see Outstanding Questions). Premature-aging diseases could represent an interesting group of disorders where aging interventions could be tested and outcomes could be determined at a much lower cost and potentially in less time. Here, treatments such as rapamycin, dietary interventions, sirtuin-activating compounds, metformin, NAD precursors, and senolytics could be more diligently tested in DNA repair disorders. A large number of therapies are emerging that may directly or indirectly lead to less DNA damage and the vast ongoing research across the globe will undoubtedly eventually be able to target this for the benefit of humankind. In sum, the future is bright.

Acknowledgments

We thank the Nordea Foundation (#02-2017-1749), the Novo Nordisk Foundation (#NNF17OC0027812), the Danish Cancer Society (#R167-A11015_001), the Independent Research Fund Denmark (#7016-00230B), the Neye Foundation, and Insilico Medicine for their great support.

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Outstanding Questions

How can we connect biochemistry with clinical phenotypes? The current biochemical knowledge of DNA repair processes is extensive with detailed mechanistic understanding of how different chemical modifications are repaired and by which enzymes. It is, however, in most cases completely unclear why mutations in different pathways yield a wide variety of phenotypes. To develop any type of intervention, extensive knowledge connecting biochemistry with clinical outcome is needed and more research should be directed towards this.

Can we use diseases displaying premature aging as models for human aging? An obstacle for interventions in aging research is that targeting aging itself is not an option for a primary outcome of a clinical trial in the current FDA setting. Here, monogenic DNA repair diseases could be targeted not only for the betterment of that particular patient group but possibly for multiple age-associated diseases.

Do small-molecule DNA repair stimulators attenuate aging? The ultimate evidence supporting a role of DNA damage and repair in aging would be the finding that stimulation of DNA repair extended the lifespan of model organisms. It would be especially compelling if it was demonstrated that the effect depends on a specific DNA repair pathway.

Does decreasing DNA repair efficacy explain variability in aging phenotypes? While evidence suggests that DNA repair declines with age, it is unclear whether there is any tissue specificity or whether the decline correlates with certain age-associated phenotypes. This is a particularly pertinent question because we need defined outcome measures in clinical trials for small-molecule DNA repair stimulators.

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